

Tech Note: Test Format and Protocol Change

PSA/SRA 57400 • *Tobacco mosaic virus* (TMV) ELISA

Technical Note

The test format and protocol for *Tobacco mosaic virus* (TMV) ELISA have changed due to exhaustion of our previous antibody supply. Our scientists worked diligently to develop, optimize and validate a new combination of antibodies with performance characteristics comparable to the previous version of the test. This ensures we will be able to supply our customers with a consistent supply of quality antibodies well into the future. The information below summarizes changes and performance characteristics to expect with the new version of Agdia's TMV ELISA. The ELISA has changed from a standard DAS ELISA to a Compound-ELISA format, utilizing a "Bottle A + Bottle B" system for the conjugate step. Antibody components from the previous Agdia TMV test are no longer available and are not interchangeable with antibody components of the new version. You can identify the previous test components from the new test components by referring to the catalog number and lot numbers; see the table below for identification purposes.

Changeover Guide

Do not use previous test components with the new test components.

Item	Previous Protocol	New Protocol
Test Format	DAS	Compound
PathoScreen® User Guide	m14	m20
Reagent Set User Guide	m16	m12
Capture Antibody	02607 and below	02608 and above
Enzyme Conjugate	03613 and below	Not used in current assay
Bottle A: Detection Antibody	Not used in prior assay	00001 and above
Bottle B: Alkaline Phosphatase Conjugated Antibody	Not used in prior assay	03614 and above
Coated Plates	00039 and below	00040 and above

For answers to your technical questions, please contact us at techsupport@agdia.com.



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Performance comparison: Cross-Reactivity & Sensitivity

Agdia's previous version of the TMV ELISA has been known for a wide cross-reactivity profile with many *Tobamovirus* species. The same is true with the new version of the assay. Optical density (O.D.) values for cross-reactivity testing shown in Table 1 represent the average of three replicates run per sample, per vendor. Each test was run per the vendor's documented protocol. For example, Agdia's ELISA was run per Agdia protocol with Agdia buffers.

Table 1: *Tobamovirus* species cross-reactivity & sensitivity comparison between TMV ELISA tests

Sample	Dilution	Agdia Previous ELISA O.D.	Agdia NEW ELISA O.D.	Vendor A ELISA O.D.	Vendor B ELISA O.D.	Vendor C ELISA O.D.
TMV	High titer	3.688	3.786	2.076	3.553	2.805
	Low titer	0.243	0.237	0.219	0.301	0.364
ToMV	High titer	3.567	3.865	0.713	0.651	3.251
	Low titer	0.164	0.307	0.167	0.195	0.203
ToBRFV	High titer	1.780	3.555	0.383	0.284	0.863
	Low titer	0.312	0.424	0.176	0.196	0.283
ToMMV	High titer	2.113	3.311	0.218	0.250	1.519
	Low titer	0.251	0.415	0.191	0.192	0.259
TMGMV	High titer	0.610	2.728	0.322	Not tested	2.851
	Low titer	0.240	0.421	0.210	Not tested	0.319
CGMMV	High titer	1.798	3.388	1.608	1.715	0.349
	Low titer	0.356	0.825	0.248	0.198	0.207
PMMoV	High titer	0.544	2.550	0.442	Not tested	3.890
	Low titer	0.234	0.674	0.167	Not tested	2.622
ORSV	High titer	1.584	3.284	0.508	Not tested	Not tested
	Low titer	0.326	1.283	0.215	Not tested	Not tested
RMV / TVCV	High titer	3.696	3.699	2.526	Not tested	2.416
	Low titer	0.460	0.556	0.330	Not tested	0.720
SHMV	High titer	2.652	3.363	1.618	Not tested	Not tested
	Low titer	0.796	1.371	0.505	Not tested	Not tested
KGMMV	High titer	0.742	2.488	Not tested	Not tested	Not tested
	Low titer	0.196	1.216	Not tested	Not tested	Not tested
BPemV	High titer	0.589	1.280	Not tested	Not tested	Not tested
	Low titer	0.406	0.565	Not tested	Not tested	Not tested
Healthy tomato leaf 1		0.128	0.111	0.145	0.202	0.187
Healthy tomato leaf 2		0.129	0.112	0.234	Not tested	0.264
Healthy cucumber leaf		0.092	0.113	0.224	0.161	0.198
Healthy pepper leaf		0.105	0.130	0.171	Not tested	0.231
Healthy orchid leaf		0.097	0.125	0.227	Not tested	Not tested
Healthy Tobacco leaf		0.126	0.107	0.261	Not tested	0.531



Recommended Seed Testing Protocol for New ELISA:

Please see Table 2 for performance of the new assay when run with common ELISA seed testing protocols. The O.D. values shown in Table 2 represent the average values of three replicates run per sample, per protocol. For seed testing, we recommend Protocol 2 or Protocol 3 as described below. Protocol 1 is not recommended due to risk of false negative results.

Table 2: Vegetable Seed Testing Protocol Comparison with new TMV ELISA version

	Protocol 1¹ (not recommended)	Protocol 2² (recommended)	Protocol 3³ (recommended)
Protocol, timing & temperature:	ISTA protocol from 7-026 ¹	Agdia protocol with overnight coating and overnight sample incubation	Agdia protocol with overnight coating and overnight sample incubation
Extraction buffer:	ISTA buffers from 7-026 ¹	ISTA buffers from 7-026 or 7-028 ²	Agdia GEB buffer
Conjugate buffer:	ISTA buffers from 7-026 ¹	Agdia conjugate buffer	Agdia conjugate buffer
Substrate buffer:	ISTA buffers from 7-026 ¹	Agdia substrate buffer	Agdia substrate buffer
Samples:	O.D. Values	O.D. Values	O.D. Values
CGMMV + cucumber seed	2.407	3.203	2.730
CGMMV + watermelon seed	3.023	3.509	3.498
ToMV + tomato seed #1	2.464	3.739	3.811
ToMV + tomato seed #2	0.348	1.485	1.379
PMMoV + sweet pepper seed	0.406	1.015	0.915
PMMoV + jalapeno pepper seed	0.206	0.742	0.853
Healthy watermelon seed	0.139	0.127	0.108
Healthy cherry tomato seed	0.145	0.179	0.128
Healthy bell pepper seed	0.099	0.136	0.116
ISTA Extraction Buffer (cuc./melon)	0.206	0.115	N/A
PBS ISTA Ext. Buffer (tom/pep)	0.109	0.128	N/A
GEB (General Extraction Buffer)	N/A	N/A	0.137

¹ Protocol 1 (not recommended): ISTA protocol with ISTA buffers from 7-026: Detection of Squash Mosaic Virus, Cucumber Green Mottle Mosaic Virus and Melon Necrotic Spot Virus in cucurbits, International Rules for Seed Testing Annex to Chapter 7: Seed Health Testing Methods.

² Protocol 2 (recommended): Agdia protocol (user guide m12 or m20) with overnight coating and sample incubation steps, ISTA extraction buffer (PBS for tomato/pepper seeds and ISTA extraction formulation for cucurbit seeds), Agdia conjugate buffer and Agdia substrate buffer.

³ Protocol 3 (recommended): Agdia protocol (user guide m12 or m20) with overnight coating and sample incubation steps, Agdia conjugate buffer and Agdia substrate buffer.

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